TRADE SECRET

Study Title

FRD-902: Acute Eye Irritation Study in Rabbits

TEST GUIDELINES: U.S. EPA Health Effects Test Guidelines
OPPTS 870.2400 (1998)

OECD Guideline for the Testing of Chemicals
Section 4 (Part 405) (2002)

EEC Methods for the Determination of Toxicity
Method B.5 Directive 92/69/EEC (1992)

AUTHOR: Carol Carpenter, B.A.

STUDY COMPLETED ON: December 14, 2007

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
DuPont Haskell Global Centers
for Health & Environmental Sciences
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Newark, Delaware 19714
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LABORATORY PROJECT ID: DuPont-24114

WORK REQUEST NUMBER: 17474

SERVICE CODE NUMBER: 602

SPONSOR: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are compatible with current OECD Good Laboratory Practices except for the items documented below. The items listed do not impact the validity of the study.

1. The test substance was characterized by the sponsor prior to the initiation of the study. Although the characterization was not performed under Good Laboratory Practice Standards, the accuracy of the data is considered sufficient for the purposes of this study. However, the test substance was characterized in compliance with Good Laboratory Practice Standards soon after the in-life phase of the study. The Certificate of Analysis is included in this report.

2. A phase inspection of the in-life portion of the study was not conducted. An inspection was scheduled for dosing the final 2 rabbits, but these animals were not dosed due to the severe ocular effects observed in the first rabbit. This type of study is routine in nature, frequently performed, and phases are routinely inspected by the Quality Assurance Unit; therefore, this deviation does not affect the validity of the study.

Study Director: Carol Carpenter 14-Dec-2001

Carol Carpenter, B.A.
Senior Staff Toxicologist

Date

QUALITY ASSURANCE STATEMENT

Work Request Number: 17474
Service Code Number: 602

Phase Audited	*Audit Dates*	*Date Reported to Study Director*	*Date Reported to Management*
Protocol:	September 20, 2007	September 20, 2007	September 20, 2007
Report/Records:	November 20, 2007	November 20, 2007	November 28, 2007

Reported by: Donna M. Johnston 14 Dec. 2007
Donna M. Johnston
Quality Assurance Auditor
Date

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Reviewed by: Susan M. Munley 14 Dec 2007

Susan M. Munley, M.A.
Research Toxicologist
Date

Issued by Study Director: Carol Carpenter 17 Dec 2007

Carol Carpenter, B.A.
Senior Staff Toxicologist
Date

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STUDY INFORMATION

Substance Tested:
- FRD-902
- 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionic acid, ammonium salt
- 62037-80-3 (CAS Number)

Haskell Number: 28308

Composition:

86%	HFPO Dimer Acid Ammonium Salt
14.58%	Water
7.0 ppm	Perfluorooctanoic acid

Purity: 86%

Physical Characteristics: Clear and colorless liquid

Study Initiated/Completed: September 20, 2007 / (see report cover page)

Experimental Start/Termination: September 24, 2007 / September 25, 2007

SUMMARY

FRD-902 was evaluated for acute eye irritation potential in 1 young adult New Zealand White rabbit. A skin irritation study was previously conducted. The test substance was not a severe skin irritant or corrosive to the skin.

An aliquot of 0.1 mL of test substance was administered to 1 eye of the animal. The treated and control eye remained unwashed following treatment. The conjunctiva, iris, and cornea of the treated eye were evaluated and scored according to a numerical scale approximately 1, 24, and 28 hours following administration of the test substance.

Brown and white discoloration of the conjunctival membrane of the treated rabbit eye, which appeared to look like necrosis, was observed at 1, 24, and 28 hours after instillation of the test substance. Corneal opacity (score of 2), iritis (score of 1), conjunctival chemosis (score of 2 or 4), and discharge (score of 2 or 3) were also observed. Fluorescein stain examinations of the treated eye were positive for corneal injury. The rabbit was euthanized the day after treatment for humane reasons.

Under the conditions of this study, FRD-902 produced ocular effects in a rabbit which appeared to look like necrosis.

INTRODUCTION

A. Objective

The objective of this study was to evaluate the eye irritation potential and the reversibility of ocular effects of FRD-902 following a single ocular application to albino rabbits.

B. Weight of the Evidence Analysis

Data relevant to the potential eye irritation/corrosivity of the test substance was evaluated in a weight-of-the-evidence analysis prior to conducting this study. This is a new and novel test substance and there is no existing human and/or animal data available for eye irritation/corrosion. The pH of the test substance was not less than or equal to 2.0 nor greater than or equal to 11.5. There are no internationally validated and accepted *in vitro* or *ex vivo* tests available for eye irritation or corrosion. The skin irritation test was conducted prior to this test. No severe irritation or corrosion was observed.

C. Principles of the Methodology

Ocular effects are evaluated and scored approximately 1, 24, 48, and 72 hours after instillation of 0.1 mL of test substance (or a weight of not more than 100 mg of test substance) into the lower conjunctival sac of 1 eye of each of 3 rabbits. The reversibility of any effects is assessed for up to 21 days, if necessary. Ocular effects are scored according to the Draize Scale (Table 1).

MATERIALS AND METHODS

A. Test Guidelines

The study design complied with the following test guidelines except as noted below:

- U.S. EPA, OPPTS 870.2400: Acute Eye Irritation, *Health Effects Test Guidelines* (1998)
- OECD, Section 4 (Part 405): Acute Eye Irritation/Corrosion, *Guideline for the Testing of Chemicals* (2002)
- EEC, Method B.5 Directive 92/69/EEC: Acute Toxicity (Eye Irritation), *Methods for the Determination of Toxicity* (1992)

The rabbit was observed at approximately 28 hours after dosing. This deviation did not impact the integrity of the study because it was an extra observation.

B. Test Substance

(Appendix A)

The test substance, FRD-902, was supplied by the sponsor. The pH of the test substance is 8. The test substance was inverted to mix before each amount for dosing was removed. The test

substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in color or physical state, was observed.

C. Test System

The young adult New Zealand White rabbit was received from Covance Research Products, Denver, Pennsylvania.

D. Animal Husbandry

1. Housing

The animal was housed singly in a stainless steel, wire-mesh cage suspended above a cage board.

2. Environmental Conditions

The animal room was maintained at a temperature of 16-22°C and a relative humidity of 30-70%. The animal room was artificially illuminated (fluorescent light) on an approximate 12-hour light/dark cycle. Any excursions outside of these ranges were of insufficient magnitude and/or duration to have adversely affected the validity of the study.

3. Feed and Water

The rabbit was offered approximately 125 grams of PMI® Nutrition International, LLC Certified Rabbit LabDiet® 5322 daily during the study. Water was available *ad libitum*.

4. Identification

The rabbit was assigned an identification number which was recorded on a card affixed to the cage. The identification number was written on the inside of the rabbit's ear with a water insoluble marker.

5. Quarantine

The rabbit was weighed and observed for general health during the quarantine period (at least 7 days).

6. Animal Health and Environmental Monitoring Program

As specified in the DuPont Haskell animal health and environmental monitoring program, the following procedures are performed periodically to ensure that contaminant levels are below those that would be expected to impact the scientific integrity of the study:

- Water samples are analyzed for total bacterial counts, and the presence of coliforms, lead, and other contaminants.
- Samples from freshly washed cages and cage racks are analyzed to ensure adequate sanitation by the cagewashers.

Certified animal feed is used, guaranteed by the manufacturer to meet specified nutritional requirements and not to exceed stated maximum concentrations of key contaminants, including specified heavy metals, aflatoxin, chlorinated hydrocarbons, and organophosphates. The presence of these contaminants below the maximum concentration stated by the manufacturer would not be expected to impact the integrity of the study.

The animal health and environmental monitoring program is administered by the attending laboratory animal veterinarian. Evaluation of these data did not indicate any conditions that affected the validity of the study.

E. Dosing, Observations, and Body Weights

Within 24 hours prior to treatment, the eyes of 1 male New Zealand White rabbit were examined using illumination, magnification, and fluorescein stain. The animal used on this study was free of pre-existing corneal or conjunctival injury or irritation and was judged to be in good health.

One rabbit was initially treated with the test substance. Since severe ocular effects were observed, no additional rabbits were treated.

An aliquot of 0.1 mL of FRD-902 was introduced into the lower conjunctival sac of the right eye of the rabbit. The left eye of the rabbit was not treated with the test substance and served as a control. The treated and control eye of the rabbit remained unwashed. The rabbit was observed for approximately 30 to 60 seconds before being returned to its cage.

Approximately 1, 24, and 28 hours after FRD-902 was administered, the rabbit was examined for evidence of eye irritation and for clinical signs of toxicity. At each of these observation periods, eyes were examined using illumination and magnification and scored for ocular reactions according to the Draize Scale (Table 1). Any other adverse ocular reactions, particularly those indicative of corrosive action, were also noted. The control eye was not scored. This untreated eye was used for comparison and was considered "normal" relative to the treated eye. Fluorescein stain examinations were conducted on the day after instillation. The rabbit was weighed on the day of treatment.

RESULTS AND DISCUSSION

Brown and white discoloration of the conjunctival membrane of the treated rabbit eye, which appeared to look like necrosis, was observed at 1, 24, and 28 hours after instillation of the test substance. Corneal opacity (score of 2), iritis (score of 1), conjunctival chemosis (score of 2 or 4), and discharge (score of 2 or 3) were also observed. Fluorescein stain examinations were positive for corneal injury in the treated eye. The rabbit was euthanized the day after treatment for humane reasons.

The ocular scores from the animal with respect to observation time are presented in Table 2. Individual body weights and clinical signs of toxicity are presented in Table 3.

CONCLUSIONS

Under the conditions of this study, FRD-902 produced ocular effects in a rabbit which appeared to look like necrosis.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, the protocol, amendments (if any), and the final report will be retained at DuPont Haskell, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware.

TABLES

Table 1
Draize[a] Scale for Scoring Ocular Lesions

(1) **Cornea**

(A) *Opacity-degree of density (area most dense taken for reading)*

No opacity	0
Scattered or diffuse area, details of iris clearly visible	1 *
Easily discernible translucent areas, details of iris slightly obscured	2 *
Opalescent areas, no details of iris visible, size of pupil barely discernible	3 *
Opaque, iris invisible	4 *

(B) *Area of cornea involved*

One quarter (or less) but not zero	1
Greater than one quarter, but less than half	2
Greater than one half, but less than three quarters	3
Greater than three quarters, up to whole area	4

(2) **Iris**

(A) *Values*

Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	1 *
No reaction to light, hemorrhage, gross destruction (any or all of these)	2 *

(3) **Conjunctiva**

(A) *Redness (refers to palpebral and bulbar conjunctiva excluding cornea and iris)*

Vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2 *
Diffuse beefy red	3 *

Table 1
Draize Scale for Scoring Ocular Lesions (Continued)

(B) *Chemosis*

No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2 *
Swelling with lids about half closed	3 *
Swelling with lids about half closed to completely closed	4 *

(C) *Discharge*

No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3

a Draize, J. H., "Dermal Toxicity." Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. The Editorial Committee of the Association of Food and Drug Officials of the United States, Austin, Texas, 1959, pp. 46-59.

* The scores marked with asterisks indicate a positive irritant effect according to U.S. EPA test guideline.

Table 2
Individual Rabbit Ocular Irritation Scores Following Treatment with FRD-902

READING	RABBIT NUMBER	CORNEA		IRITIS	CONJUNCTIVA				FLUORESCEIN STAIN
		OPACITY	AREA		REDNESS	CHEMOSIS	DISCHARGE	OTHER	
1 HOUR	279	2	4	0	0	2	3	-	NA
24 HOURS	279	2	2	1	0	4	2	*	POS
28 HOURS	279	2	4	1	0	4	2	*	POS

POS= positive for corneal injury
NA = not applicable
- = No other effects
* = Brown and white discoloration of the conjunctiva

Table 3
Body Weights and Clinical Signs of Toxicity

RABBIT NUMBER	SEX	INITIAL WEIGHT (g)	FINAL WEIGHT (g)
279	Male	2846	*

* No final body weight collected.

No clinical signs of toxicity were observed in the rabbit during the study.

APPENDIX

Appendix A
Certificate of Analysis

E. I. du Pont de Nemours and Company
Wilmington, DE 19898
USA

CERTIFICATE OF ANALYSIS

This Certificate of Analysis fulfills the requirement for characterization of a test substance prior to a study subject to GLP regulations. It documents the identity and content of the test substance. This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 792).

Haskell Code Number	H-28308
Common Name	HFPO Dimer Acid Ammonium Salt
Purity Percent	86%
Other Components	Water – 14.58% Perfluorooctanoic acid – 7.0 ppm
Date of Analysis	October 4, 2007
Recommended reanalysis interval	1 year
Instructions for storage	NRT&H
Reference	DuPont-24003
Analysis performed at	E. I. DuPont de Nemours and Company DuPont Haskell Laboratories Newark, Delaware USA

Approver:

Peter A. Bloxham, Ph.D.
Senior Research Chemist

18-OCT-2007
Date